REVIEW

Gene therapy for coronary restenosis: is the enthusiasm justified?

M O'Sullivan, M R Bennett

Despite dramatic technological advances in coronary intervention, restenosis following percutaneous coronary intervention remains an important cause of morbidity with major financial implications.[1] By six months postprocedure, some 16–32% of highly selected patients receiving optimal treatment within the privileged context of a clinical trial have developed restenosis, necessitating target vessel revascularisation in 9–15% of patients. The development of antirestenotic treatments is therefore an area of intense research activity. The failure of conventional pharmacological agents to inhibit restenosis, along with concern over the long term safety and efficacy of intracoronary brachytherapy, has fostered the belief that gene therapy may be the future of antirestenotic treatments.[2 3] Furthermore, the focal nature of restenosis makes it a highly attractive target for locally delivered genetic material that may have toxic effects if administered systemically. However, the important question remains: can research in this field translate into clinically useful treatment or is our enthusiasm for antirestenotic gene therapy misplaced?

There are two fundamental questions that must be answered before antirestenotic gene therapy may become a reality. Firstly, what genetic material should be delivered? We believe that current understanding of the pathogenesis of human restenosis is insufficient to allow us to answer this question confidently. Secondly, and equally important, how should genetic material be delivered effectively and safely?

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Targets for gene therapy

DIFFERENCES BETWEEN ANIMALS AND HUMANS

Many of the proposed targets for antirestenotic gene therapy have arisen from study of the effects of vascular injury in animals. Myriad papers report delivery of various transgenes that suppress the response to experimental vascular injury. This often leads to the postulate that a similar strategy would limit human restenosis. However, for such "gene therapies" to be useful in humans, the animal vascular injury and human percutaneous coronary intervention must produce similar physical insult to the vascular wall and provoke a similar injury response. Unfortunately, we cannot be certain that this is the case.

The most frequently used model for restenosis is balloon overstretch injury to rodent, rabbit, or pig arteries, both peripheral and coronary. Although balloon injury to animal vessels and human percutaneous coronary intervention both evoke a healing response, certain fundamental differences may limit the applicability of balloon injury models to humans. Firstly, angioplasty is invariably performed in humans at the site of obstructive atherosclerotic disease. In most animal studies, balloon injury is inflicted upon a previously undamaged vessel. Both the response to injury and the ability to deliver treatment locally are profoundly different in the diseased and the normal artery. Attempts have been made to model more closely the human atherosclerotic artery by producing a "plaque" at the site of balloon injury through cholesterol feeding with or without previous injury. Unfortunately, the resulting lesions may not respond similarly to human atheroma. Secondly, the rat and rabbit models of vascular injury, which account for the majority of the literature in this field, produce endothelial denudation and longitudinal traction or torsion, an insult that differs considerably from the endothelial and medial damage following human percutaneous coronary intervention. The models of vascular injury that most closely resemble the injury of human angioplasty are porcine coronary overstretch injury and coronary angioplasty in the nonhuman primate. These procedures produce a deep medial injury and both models enable the study of effects of coronary stenting at the time of injury. However, for logistical reasons, neither model has been widely used. Finally, the histological consequences of animal vascular injury tend to be analysed up to 28 days from surgery, well before the emergence of human restenosis.

BIOLOGICAL PROCESSES INVOLVED IN RESTENOSIS

The neointimal response to injury is known to vary among animal species and even strains of the same species. Thus, the mechanism of response to vascular injury in humans may well differ from that in other species. However, two major components that generate restenosis are neointimal formation and negative remodelling. Neointimal hyperplasia following experimental injury involves a combination of many processes, including vascular smooth muscle cell (VSMC) and adventitial cell migration and

proliferation, and matrix deposition. Negative remodelling may also arise from many processes, including VSMC apoptosis, medial and adventitial fibrosis, and matrix remodelling.[4] The key regulators of each of these processes have been genetically manipulated in various animal models (table 1).

It has been well shown that genetic strategies that limit cellular proliferation following experimental injury inhibit intimal hyperplasia.[5] Thus, local delivery of agents that inhibit expression of either immediate early genes or promoters of cell cycle transit diminish neointimal formation. A similar effect is achieved through induction of negatively acting cell cycle regulators. An alternative means of limiting VSMC accumulation after experimental vascular injury is through overexpression of proapoptotic factors. Much recent work has focused on the role of matrix deposition and turnover, as regulated by matrix metalloproteinases, and their inhibitors (tissue inhibitors of matrix metalloproteinase), in neointima formation.[6] However, genetic manipulation of expression of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinase has provided results that are contradictory.

Another potential target for gene therapy identified through experimental models is the endothelium. Local overexpression of endothelial or inducible nitric oxide synthase inhibits neointimal formation through multiple effects including inhibition of VSMC migration and proliferation, promotion of VSMC apoptosis, and inhibition of matrix remodelling.[7] The possibility that localised overexpression of vascular endothelial growth factor may limit restenosis has been raised by the observation that delivery of recombinant vascular endothelial growth factor to the site of rabbit iliac stenting accelerates re-endothelialisation and inhibits in-stent neointimal formation.[8]

The relevance of these studies to human restenosis is determined by the closeness with which the human response to coronary injury replicates that within animal models. Although intimal hyperplasia is involved in restenosis following coronary angioplasty without stenting, constrictive remodelling plays a much more important part.[9] Thus, therapeutic strategies aimed at reducing intimal hyperplasia alone are unlikely to be sufficient to prevent restenosis following angioplasty. Constrictive remodelling is effectively eliminated by coronary stenting, a procedure now used in at least 70% of coronary interventions, and in-stent stenosis is therefore predominantly caused by neointimal formation. The in-stent neointima consists of VSMCs embedded within a dense extracellular matrix[10]; however, the contribution of cellular proliferation to neointimal formation is debated. While pockets of VSMCs expressing proliferative markers have been observed at sites of peripheral in-stent stenosis, this is not the case for coronary in-stent stenosis. Indeed VSMCs cultured from sites of human coronary in-stent stenosis proliferate less readily than normal medial cells.[11] Although local delivery of the antiproliferative rapamycin at the time of coronary stenting reduces human in-stent stenosis,[12] this effect may be caused by mechanisms other than inhibition of VSMC proliferation. While the use of gene therapies directed against cellular proliferation may prove useful in the battle against restenosis, it cannot be assumed that a purely antiproliferative strategy would be sufficient. Indeed, the pleiotropic nature of the reaction to injury argues against manipulation of a single molecule in a single biological process.

An alternative, or adjunctive, approach would be the delivery of proapoptotic genetic material. This may, however, prove to be a high risk tactic—the promotion of VSMC apoptosis may promote plaque destabilisation.[13] Similar concerns may be raised about the use of genetic strategies that modulate matrix turnover through manipulation of expression of matrix metalloproteinases or their tissue inhibitors. The complex matrix reaction to human vascular injury is poorly understood and inhibition of neointimal formation by this means may be at the expense of plaque stability.

Table 1 Summary of targets for gene therapy in animal models of vascular injury

	Model	*Lesion inhibition (%)*
Antiproliferative action		
Antisense oligodeoxynucleotide		
c-*myb*	Rat carotid	84
c-*myc*	Porcine coronary	70
PCNA	Rat carotid	80
cyclin B	Rat carotid	60
cyclin G_1	Rat carotid	64
CDK-1	Rat carotid	40–47
CDK-2	Rat carotid	55–60
CDK-1 + CDK-2	Rat carotid	85
CDK-1 + PCNA	Rat carotid	60
CDK-1 + cyclin B	Rat carotid	78
Decoy oligodeoxynucleotide		
E2F	Rat carotid	74
Gene transfer		
pRb (non-phosphorylatable)	Rat carotid	50
	Porcine femoral	47
p130	Rat carotid	81
$p21^{Cip1}$	Rat carotid	46–58
	Porcine femoral	37
$p27^{Kip1}$	Rat carotid	49
p53	Rabbit carotid	85
GAX	Rabbit iliac	56
ras (dominant negative)	Rat carotid	62
Pro-apoptotic action		
Antisense oligodeoxynucleotide		
bcl-x_L	Rabbit carotid	56*
Gene transfer		
Fas-ligand	Rat carotid	60–73
Matrix action		
Gene transfer		
TIMP-1	Rat carotid	30
Pleiomorphic action		
Gene transfer		
eNOS	Rat carotid	56–72
iNOS	Rat carotid	95
	Porcine iliac	52

CDK, cyclin dependent kinase; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; GAX, growth arrest specific homeobox gene; pRb, retinoblastoma protein; PCNA, proliferating cell nuclear antigen;TIMP, tissue inhibitor of metalloproteinase.
*Regression of previously formed lesion, as opposed to inhibition of lesion formation.

Delivery of gene therapy

If the ideal antirestenotic transgene were identified, its use would depend on efficient local delivery to the vessel wall, where cellular uptake would lead to prolonged expression. Many catheter systems are available for local intravascular gene delivery at the time of percutaneous coronary intervention. However, cellular uptake is limited by the poor permeability of atheroma, which is rich in lipid and

connective tissue. While the use of needle catheters may facilitate intraluminal gene transfer, this technology has not been fully evaluated and may exacerbate vascular injury. Improvements in coated stent technology may provide a more ideal platform for local intravascular gene delivery; however, few genetic studies using stent-based delivery have been performed.

In addition, we still lack a synthetic, non-immunogenic and targetable gene transfer vector.[2] Both plasmid liposomes and retroviruses are limited by low gene transfer efficiency and retroviruses have the associated theoretical risk of transformation. Adenoviruses, which provide high gene transfer efficiency and can transduce quiescent cells, have been the main focus of research in this field. However, even second generation adenoviruses provoke a local inflammatory response, cause only transient transgene expression, and may not be effective in humans who are preimmunised by contact with native adenovirus.[14] Furthermore, there has been justified concern about the safety of gene therapy using adenoviral vectors,[15] particularly in diseases such as restenosis that are rarely lethal.

Conclusions

We propose that the identification of a suitable target for antirestenotic gene therapy requires considerable basic scientific progress in the study of *human* restenosis, not just animal models. Indeed, the complexity of restenosis renders identification of individual processes and single molecular targets difficult, and the likelihood that manipulation of a single gene product would inhibit restenosis is low. Much optimism within the field of vascular gene therapy has been based on uncontrolled trials and anecdotal cases. Such enthusiasm should be tempered by acknowledgement of the deficits within current understanding of the mechanisms of restenosis and the technology required to deliver genes to diseased human vessels.

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